Fass, D. N. Isolation of γ-amylase (glucamylase) from the culture filtrate of N. crassa.

An amylase-super-producer strain, e.g., inos (89601) a, (FGSC#498) is grown 84-96 hours in 1% sucrose Vogel's medium. Good yields may be expected from 1 liter cultures in 2.8 l Fernbach flasks incubated at 25°C on a shaker. Adequate aeration is essential for production of the enzyme. At the end of this time, the mycelia are removed by filtration and the medium is chilled to 4°C and cold ethanol is added to 40%. The solution is allowed to stand overnight at 4°C ond the resulting precipitate is removed by centrifugation at 25000 X g for 10 min. To the alcoholic supernatant is added a water solution of 2% glycogen in the proportion of 25 ml/l of original medium. The white precipitate which forms is centrifuged immediately at 4000 X g for 10 min ond redissolved in a small volume of Vogel's salts. This mixed enzyme solution is incubated for 1 hr at 37°C and then dialyzed twice at 4°C against 50 vols of citrate buffer 0.01 M in Na^+, pH 5.0, for 4 hrs and 8 hn. The da ze sample is applied to a 2 x 15 cm column of Amberlite CG-50 equilibrated with the some buffer. Elution is carried out at 4°C with a 500 ml linear gradient from 0.01 to 1. 1 N Na^+ at approximately 40-50 ml/hr. Citrate is the counter-ion.

The amylolytic activity recovered at about 0.4 N No+ shows an E-fold increase in specific activity, no invertase or α-amylase activity, and a single bond in acrylamide gel electrophoresis. This work was supported in part by the NSF and NIH Training Grant in Genetics (T01-GM01316) to Florida State University. ■ ■ -Genetics Laboratories, Department of Biological Science, Florida State University, Tallahassee, Florida 32306.